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Gametogenesis and Spawning in a Population of *Macoma balthica* (Pelecypoda: Tellinidae) from Long Island Sound

by

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Abstract. A population of *Macoma balthica* in Long Island Sound, Stonington, Connecticut was studied for 24 months during a 4-yr period to determine the sequence of gametogenic development of gonadal tissue and the frequency and duration of the spawning cycle under natural conditions. This population was observed to spawn annually in March–April. Sexes were distinguishable in all size classes studied, except those individuals in an "inactive" condition. A low incidence of simultaneous hermaphroditism suggests that *M. balthica* is a stable gonochoric species. There was no evidence of protandry. Females predominated in the population in the ratio of 9:11. Photomicrographs of the gametogenic cycle of both male and female clams are included.

INTRODUCTION

The circumarctic bivalve *Macoma balthica* (Linnaeus, 1758) is widely distributed, common to littoral and shallow sublittoral habitats in boreal and temperate waters of both Europe and North America. Most studies of *M. balthica* reproduction have been done on European (Caddy, 1967; Lammens, 1967; von Oertzen, 1972) and Canadian (Sullivan, 1948; Lavoie, 1970) populations. Other studies of North American populations were carried out in Buzzards Bay (Gilbert, 1978), Chesapeake Bay (Shaw, 1965), and San Francisco Bay (Nichols & Thompson, 1982).

Macoma balthica is dioecious; the sexes can be conclusively distinguished only after examination of the gonads. *Macoma balthica* apparently has a single annual spawning season in all populations studied except in the Chesapeake Bay population, where a biannual cycle of larval settlement was reported (Shaw, 1965).

In an attempt to define more clearly the latitudinal patterns of reproduction in this species, a population of *Macoma balthica* from Stonington, Connecticut, was studied to determine (1) the age of maturation and occurrence of gametogenic development and (2) the frequency of spawning. This paper presents the results of that 4-yr study of the breeding habits of this species in Long Island Sound.

MATERIALS and METHODS

Specimens of *Macoma balthica* were collected from an intertidal sandflat located at Barn Island in Stonington, Connecticut (41°20′N, 71°53′W). Monthly collections were made from April to August 1982, November to April 1982–1983, June to March 1983–1984, and February to April 1985. Sample sizes varied from 12 to 112 clams, which were 10.1 to 27.3 mm in shell length. A total of 740 clams were examined and used in the analysis of the reproductive cycle.

The clams were counted and individual maximum lengths (±0.1 mm) were measured. The visceral mass was removed and fixed in 10% buffered formalin. The tissues were then prepared for histological examination (Brousseau, 1978). A microscopic examination was made to assign each individual to the appropriate category of gonadal condition. There was evidence of seasonal changes in gonadal color as reported by Lammens (1967) and Caddy (1967). When ripe, ovaries appear gray to gray-orange in color and the testes are white. Mean oocyte diameter was estimated for a sample of 20 females reported in a "ripe" gonadal condition.

The reproductive condition of the clams was measured by stereology, a procedure adapted by Bayne *et al.* (1978), Newell *et al.* (1982), and Brousseau (1983) for mussels. This method is based on a procedure referred to as point-

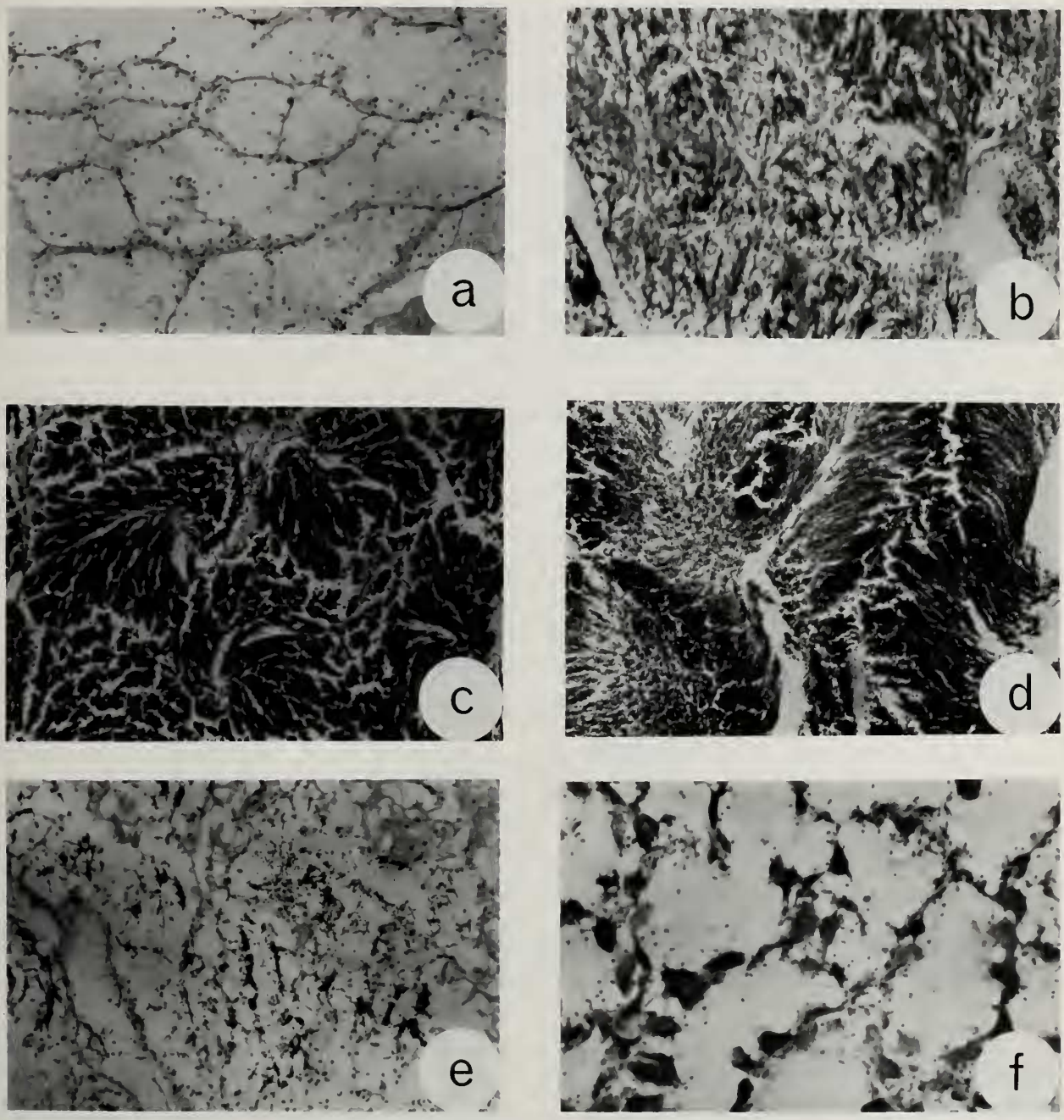

Figure 1

Photomicrographs of gonadal stages of male and female *Macoma balthica* at 125× magnification. a, indifferent male or female, 27 May 1982. b, developing male, 6 November 1983. c, ripe male, 26 April 1982. d, spawning male, 15 April 1983. e, spent male, 26 April 1982. f, developing female, 4 December 1983.

counting volumetry, which is accomplished by superimposing a regular point lattice on the tissue section and counting the points that lie on transections of the sex cells (Weibel *et al.*, 1966). The proportion of gonadal tissue consisting of follicles containing developing or ripe gametes is reported as the "gamete volume fraction" (GVF). For any individual clam, the GVF can vary between zero, for a reproductively inactive clam, and one, for a clam showing maximal reproductive development. The monthly mean GVF represents the mean of 10 estimates of the

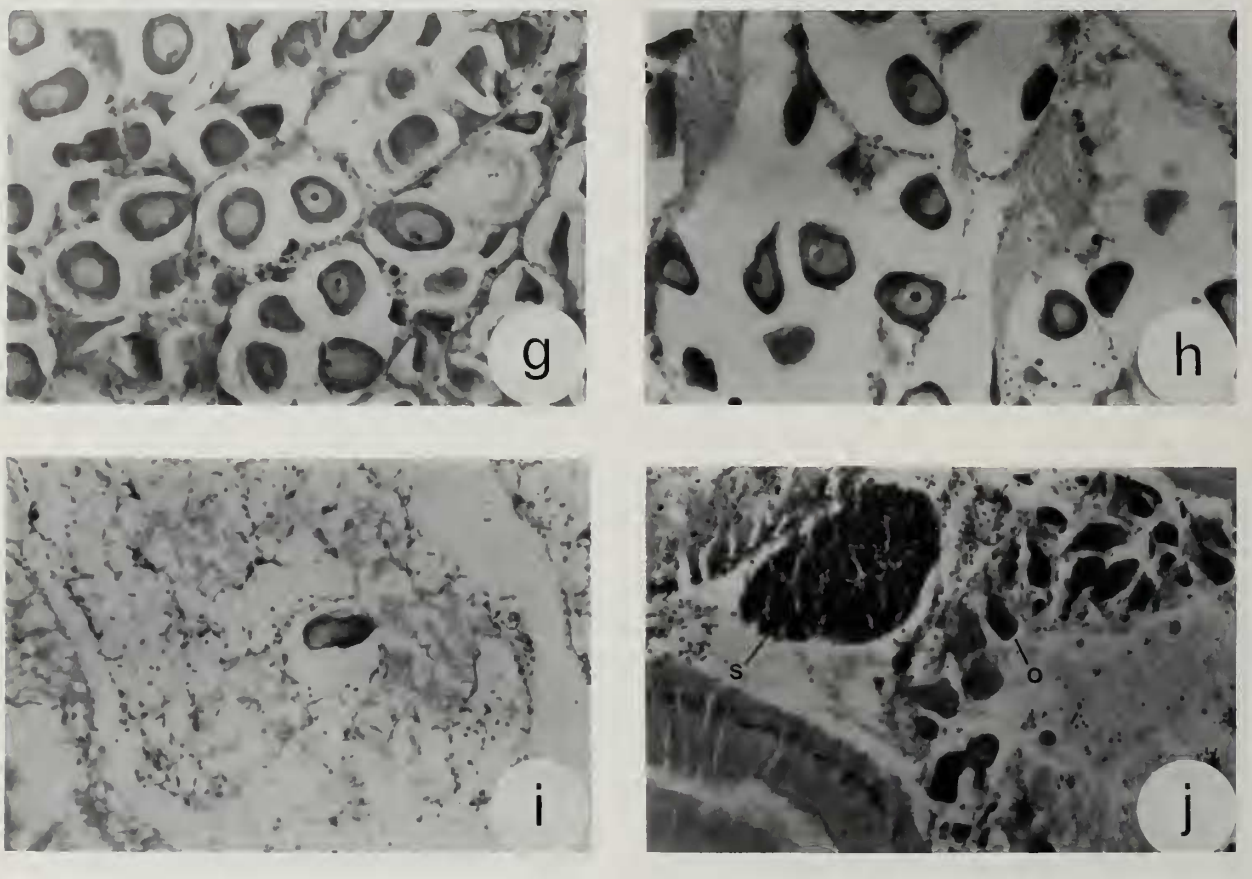

Figure 1 (continued)

g, ripe female, 15 April 1983. h, spawning female, 26 April 1982. i, spent female, 27 May 1982. j, hermaphrodite, 15 February 1984.

GVF from each clam sampled. The number of clams included in the estimate varied from 9 to 109. These proportions were then arcsine transformed and the variance for each monthly GVF was calculated.

RESULTS

Categories of Gonad Condition

The following descriptions of the male and female developmental stages represent and attempt to divide the reproductive process (either spermatogenesis or oogenesis) into distinct phases. The criteria used are based solely on morphological observations. Categories comparable to those already in use for other species (Ropes & Stickney, 1965; Brousseau, 1978, for *Mya arenaria*; Porter, 1964; Keck *et al.*, 1975, for *Mercenaria mercenaria*; Brousseau, 1981, for *Petricola pholadiformis*; Brousseau, 1982, for *Geukensia demissa*) have also been used in the study where appropriate.

Developmental Stages of the Male

Indifferent stage: The interfollicular space dominates and consists almost entirely of large vacuolated nutritive cells with a few spermatogonia scattered along the periphery or near the central axis. There were no pycnotic cells nor multinucleated non-pycnotic cysts apparent in the follicles (Figure 1a).

Developing stage: The spermatogenic cells begin to proliferate around the follicle walls and between the follicular cells within the developing follicle. Maturation of spermatozoa is most rapid in the center of the follicle. Gradually the follicle breaks down and spermatozoa developing in the middle of the follicle are joined by those arising from the periphery (Figure 1b).

Ripe stage: The mass of mature spermatozoa increases in volume and the individual cells arrange themselves in bands, with tails pointing toward the center of the lumen (Figure 1c).

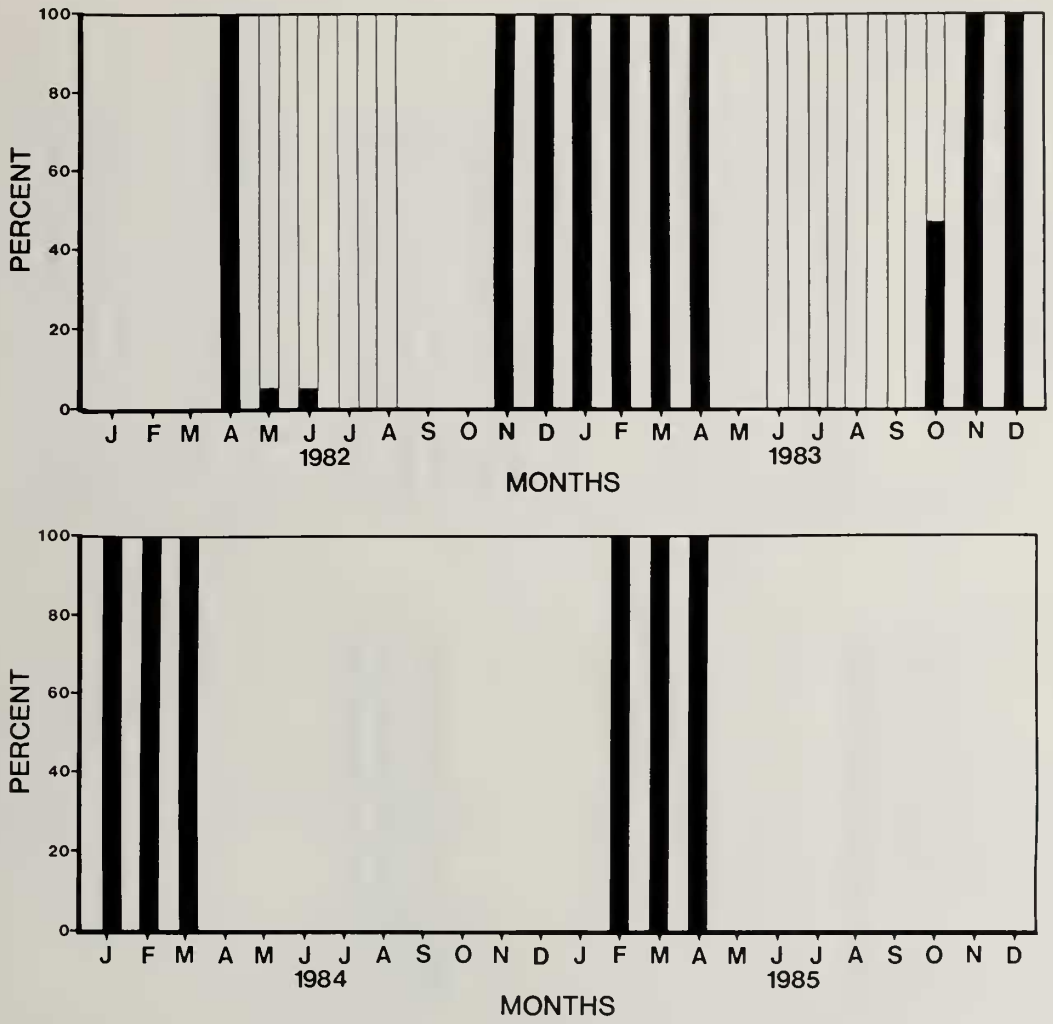

Figure 2

Percentage of *Macoma balthica* population with active or inactive gonads during 1982–1985. Open portions of each represent inactive gonads (indifferent or spent); solid portions represent active gonads (developing, ripe gametes or partially spawned). Observations on both males and females are combined.

Partially spawned stage: A marked decrease occurs in the number of spermatozoa filling the lumen with most follicles emptying or empty (Figure 1d).

Spent stage: In totally spawned males a few residual sperm are visible but the majority of follicles are empty. Spermatocytes are rare (Figure 1e).

Developmental Stages of the Female

Indifferent stage: The interfollicular space dominates and consists almost entirely of large vacuolated cells. The follicles are empty except for occasional residual free oocytes (Figure 1a).

Developing stage: Oocytes become more noticeable along the follicle walls, increasing in size and number. The developmental phase is a continuous process, involving a proliferation and maturation of the oocytes, with an accompanying reduction in interfollicular connective tissue. The developing oocytes, which begin as hemispherical or cylindrical cells attached to the wall of the follicle, become enlarged spherical cells 30 to 40 μm in diameter as maturity approaches (Figure 1f).

Ripe stage: Ripe females are characterized by large, round oocytes, 65 to 70 μm in diameter, some of which are attached to the follicular wall by slender stalks. Others are free oocytes in the lumen of the follicle. A prominent ec-

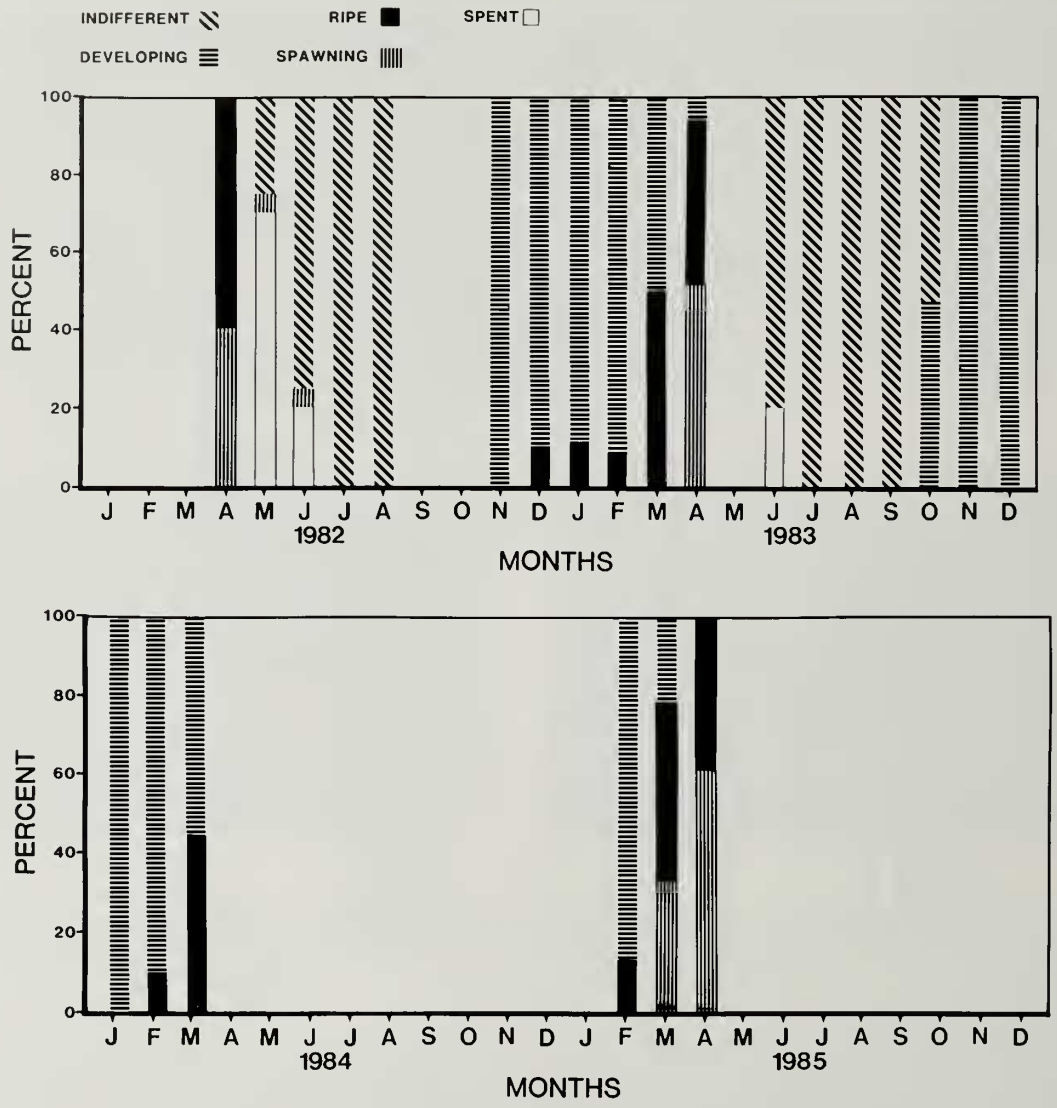

Figure 3

Percentages of *Macoma balthica* with gonads in each developmental phase during 1982–1985. Values for males and females are combined.

centrically placed nucleolus is visible within the nucleus (Figure 1g).

The number of ripe oocytes decreases in the lumen, and some follicles completely lack gametes (Figure 1h).

Spent stage: Clams that have recently undergone oogenesis can be recognized by the presence of a few unspawned oocytes in the lumen. These may be in various degrees of cytolysis. Resumption of oogenic activity may be evident in some individuals (Figure 1i).

Reproductive Cycle

Reproductively active individuals (developing, ripe, and spawning) were encountered throughout the 4-yr study period, except in June through September. The largest numbers of active individuals occurred in November through April (Figure 2). Gametogenesis began in October each year in both sexes, but fully ripe individuals did not appear until December in 1982 and February in 1984 and 1985 (Figure 3). Spawning individuals were first observed in April of 1982 and 1983 and in March of 1985. Discharge of eggs was completed by early summer. By mid-May over 90% of the clams had completely spawned or had returned to the indifferent condition.

The GVF values for male and female *Macoma balthica* from this population are shown in Figure 4. The pattern of GVF values and the maximum GVF attained were similar during each year of the study. The post-spawning

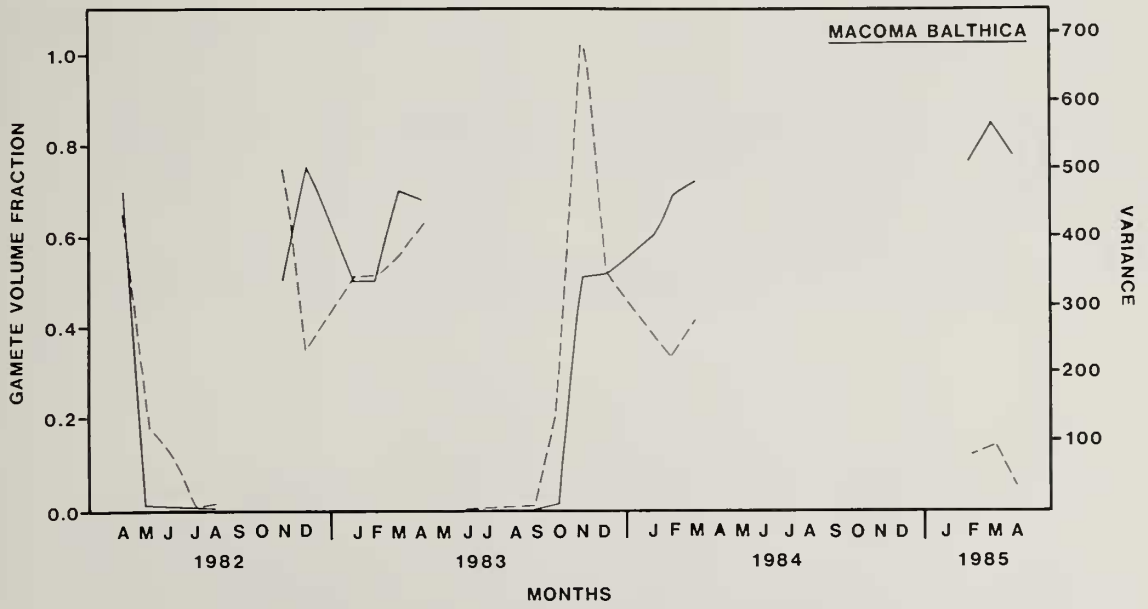

Figure 4

Mean gamete volume fractions (solid line) and variance (dotted line) for *Macoma balthica*. Values for males and females are combined.

minimum GVF values occurred in June through September. Increasing GVF values in November were due to the onset of gametogenesis. Peak GVF values of 0.85 were observed in March 1985.

Variance in GVF during each sampling period provides a measure of the intrapopulation synchrony of the reproductive cycle. The larger the variance, the greater the variability in the gametogenic condition of individuals during that sampling period. In general, the clams were least closely synchronized (*i.e.*, had the highest variance) during the fall and winter months, indicating that the clams were ripening at different rates. This increased variance continued through the spawning period, suggesting that spawning occurred at different times during a 2-month period.

In the Stonington population, the proportion of females in all size classes (n = 589) differed significantly from one-half (Table 1). Male and female gonads were distinguishable in all size classes studied (>11.0 mm). Although no protandry was observed, there was evidence of simultaneous hermaphroditism in two individuals (Figure 1j). Hermaphrodites were collected in February and March and appeared to be undergoing normal gametogenic development.

Table 1

Proportion of females in each size class studied in the population of *Macoma balthica* from Stonington, Connecticut. The number of individuals per sample is given in parentheses. Year classes were determined using external growth annuli (Brousseau, unpublished). *$P < 0.05$.

Size class (mm)	Year class	Proportion females	Confidence limits (95%)
11.0–14.9	2	0.533 (15)	0.266–0.787
15.0–17.9	3	0.510 (96)	0.406–0.613
18.0–19.9	4	0.625 (152)	0.539–0.696*
20.0–21.9	5	0.534 (161)	0.451–0.608
22.0–22.9	6	0.515 (68)	0.388–0.631
23.0	6	0.536 (97)	0.426–0.632
Total		0.552 (589)	0.509–0.590*

DISCUSSION

At Barn Island, the population of *Macoma balthica* shows a single annual spawning period which occurs during the spring. This pattern coincides with those reported for *M. balthica* throughout most of its geographic range (see GILBERT, 1978 for a review). Of the three studies in which multiple spawnings were reported, however, only that by SHAW (1965) points to the occurrence of more than one major gametogenic cycle during the year. The work by BATTLE (1932) and CADDY (1967) suggests that one or more pulses of gamete release may occur during spawning in this species.

As a rule, North Atlantic species of bivalves spawn during the warmer months of the year (SASTRY, 1979) and ripening of gametes begins in the spring. One unusual feature of the reproductive cycle of *Macoma balthica* is that gonadal development occurs only during the winter months.

This is probably due in part to the feeding behavior of *M. balthica*. Unlike most other species of bivalves, which feed on plankton, *M. balthica* is primarily a deposit feeder. Hence, its food source is available throughout the year.

Macoma balthica is dioecious; the sexes are distinguishable either by examining the sex products, or from inspection of gravid individuals. Ovaries are orange when gravid, whereas testes are cream colored. Although few species of bivalves can be sexed in this manner, there are some exceptions, the most notable being the blue mussel *Mytilus edulis* (CAMPBELL, 1969). The low incidence of hermaphroditism exhibited by *Macoma balthica* suggests stable gonochorism, a condition characterized by the presence of some hermaphrodites in a normally gonochoristic species.

Information on sex ratios in this species shows little agreement among populations. The proportion of females in the population studied here is slightly greater than one-half (Table 1). In a population in the Thames River, England, males predominated in a ratio of 9:7 (CADDY, 1967). Caddy attributed this to a preponderance of 2-yr old males undergoing protandric development (a primary male phase). On the other hand, a population in Falmouth, Massachusetts (GILBERT, 1978) was reported to have a 50:50 sex ratio. The large differences in sex ratios among populations of *M. balthica* suggest either that patterns of sexual differentiation vary from population to population or that more information is needed before generalizations can be made.

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